

## THE DEVELOPMENT OF CHEMOSENSORY ATTRACTANTS FOR BROWN TREE SNAKES

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### Abstract

Concern that the brown tree snake (*Boiga irregularis*) may be inadvertently transported in cargo shipments from Guam has resulted in management programs aimed at limiting the likelihood of snake stowaways. A primary tool used to capture snakes is a trap within which is a live mouse lure. Because using live mice presents logistical problems and concerns about animal care and use, it is desirable to develop an effective inanimate lure. Previous studies indicate that brown tree snakes are attracted to carrion odors. Here, we present the results of several pilot studies examining the attractiveness of cadaverine, dimethylamine, dimethyl disulfide, dimethyl sulfide, ethanethiol, trimethylamine, and putrescine (all components of carrion odor) to brown tree snakes. Results indicate that the major components of carrion odor, as defined by human perception, are not necessarily salient odors to brown tree snakes. We encourage a more systematic approach to the isolation of specific brown tree snake attractants by testing serial fractions of carrion odor for bioactivity.

### 1. INTRODUCTION

Since arriving on Guam in the late 1940's or early 1950's, the brown tree snake (*Boiga irregularis*) has virtually extirpated the island's avifauna (Savidge 1987, Fritts 1988). There is concern that the snake may further extend its range via incidental transport in cargo and cause similar ecological disruptions elsewhere. The Department of Agriculture and Wildlife Resources of Guam, the Biological Resources Division of the U. S. Geo-

logic Survey, and the U. S. Department of Agriculture's Wildlife Services Program have all implemented research and control programs aimed at minimizing the risk of brown tree snakes being accidentally transported in cargo. Control efforts largely focus on creating "snake-free" zones in the areas adjoining cargo and areas targeted as sites for the preservation and reintroduction of endemic species of avifauna.

A variety of control methods have been explored and implemented on Guam to date. The Wildlife Services program uses trained Jack Russell terriers to identify cargo containing brown tree snakes. Barrier systems have been developed that decrease the likelihood of snakes crossing over protected areas (Campbell 1996). Fumigants for treatment of outward bound cargo have been tested. However, due to the cost and logistical difficulty of fumigating all cargo, fumigants have not been implemented as a general control strategy (J. E. Brooks & P. J. Savarie, unpublished data; P. J. Savarie, J. E. Brooks & Wood, unpublished data). Similarly, studies investigating the usefulness of dermally delivered toxicants have been performed, but a safe and long lasting snake toxicant and toxicant delivery system has not been developed (J. E. Brooks, P. J. Savarie & J. J. Johnston, unpublished data). Nighttime Surveys, during which perimeter fences around cargo areas and airports are patrolled, are a part of normal snake control operations; snakes are individually removed from fences during the patrols. Perhaps the most effective method, but also the most labor intensive, has been the Wildlife Services program of trapping snakes, thus intercepting snakes before they can slither into cargo areas (Linnell, Engeman, Pitzler, Watton, Whitehead & R. C. Miller, in press; U.S. Dept. Agric. 1996).

The lures used for capturing snakes are live mice placed within holding compartments within traps. Maintaining mice imposes logistical constraints and the cost of maintaining mice in traps is high because mice must be fed and watered on a continual basis. More traps could be set if an artificial lure were used, and improving the efficiency of trapping efforts has remained a high priority. Also, animal welfare concerns reduce the desirability of using a live animal lure. Thus, there is a clear need for the discovery, development, and implementation of a highly effective inanimate lure for brown tree snakes.

Identifying the specific cues which stimulate appetitive foraging behavior in snakes will enable the efficient development of an effective inanimate lure. Various snakes, including the brown tree snake, may use visual and/or chemical cues to locate distant prey (Eichholz & Koenig, 1992; Fritts, Scott & Smith, 1989; Neal, Montague & James, 1993; Rodda, 1992). For the brown tree snake, the cues that are reported as dominant vary between studies. Chiszar, Kandler & Smith (1988) noted that visual cues alone would elicit attack behavior and that visual cues are important because if a lure container is visibly empty, attractive effects of chemical cues may be lost (Chiszar, 1990). However, Fritts et. al (1989) showed that brown tree snakes will enter traps baited only with bird odors (bird cage litter).

Lately, efforts to identify chemical lures for brown tree snakes have focused on reproductive signals (M. J. Greene & R. T. Mason, unpublished data) and cues from prey (Fritts et. al 1989; Shivik & Clark, 1997; Shivik, 1998). Relevance of behavioral assays to field applications is clearly an important issue. For example, laboratory investigations typically use the frequency of tongue flicks as a metric of interest by snakes for a specific chemical stimulus (Burghart, 1969). The threshold for this assay is low and many chemical stimuli are identified as candidate lures in the laboratory, only to be discarded as ineffective lures in the field (G. H. Rodda & D. Chiszar, unpublished data). In the continuum of appetitive foraging behaviors, the tongue flick is a low-effort, low-cost behavior of snakes that has utility as an information gathering tactic.

The tongue flick, therefore, is a questionable behavioral index for evaluating a snake's willingness to pursue prey, and it is not an appropriate index of a snake's prob-

ability of entering a trap. Shivik (1998) showed that orientation behavior, i.e., directional probing of the head, was an easily monitored behavior that gave good concordance between laboratory studies and field trapping data. Orientation and probing behaviors presumably require higher motivational levels in the appetitive foraging process, and thus better reflect the salience of a stimulus. Most importantly, however, orientation behaviors are consistent with the behaviors required for a snake to enter a trap during the appetitive foraging process. Studies aimed at developing effective lures for brown tree snakes must use a metric that is directly applicable to the problem of trapping brown tree snakes. Appropriate methods include recording duration of orientation behavior of snakes in the laboratory or measuring capture rates on Guam. One objective of this paper is to examine possible lures using trap success as a basic metric.

Earlier studies showed that both visual and odor cues were important for investigatory behavior of live prey by brown tree snakes (Shivik, 1988). The combination of cues produced more intense investigatory behavior and higher trapping success than when either component was tested alone. Other studies showed that carrion was a suitable lure (Shivik & Clark, 1997). In contrast to live prey lures, carrion odor alone (i.e., with no visual prey stimulus) was sufficiently potent, yielding trap success equivalent to that using live mouse lures. Actual carrion is not a useful lure because mice rot away too fast in field conditions to enable efficient long-term trapping. Therefore, we set out to identify salient components of carrion odor with the goal of developing an effective inanimate lure. Initial efforts at identifying attractive stimuli, the subject of this report, focused on amine and sulfur compounds associated with the decomposition of flesh.

## 2. METHODS

We hypothesized that compounds that attract brown tree snakes could be those that are considered major components of the odor of carrion. These include cadaverine, dimethylamine, dimethyl disulfide, dimethyl sulfide, ethanethiol, trimethylamine, and putrescine, (Eskin, Henderson & Townsend, 1971; Stager, 1964). For testing, we chose odor concentrations that were easily detectable, but not overpowering, to the human nose.

We performed trapping experiments on Guam to determine if compounds characteristic of decomposition are attractive to brown tree snakes. All traps were cleaned with cool, high-pressure water and soaked in a cold-water bleach solution (10% chlorine bleach) for more than one hour to minimize extraneous odors. Trap-lines were set along forest edges with control and treatment traps randomly distributed within each trap-line. Traps were hung approximately 1.5 m high in trees. Differences in trapping rates were assessed using analysis of variance, and multiple comparisons were made using the Tukey test. These experiments were reviewed and approved by Colorado State University and National Wildlife Research Center Animal Care and Use Committees.

In the first experiment, dimethylamine, trimethylamine and cadaverine were tested. We used 20 ml of a 20% solution of each chemical, diluted them to 200 ml in a spray bottle and sprayed the solution onto fur-covered mouse models (commercially available cat-toys). Four traps containing the soaked models and five traps containing live mice were placed in traps above Haputo Beach during August, 1996. This trap-line was run for two nights and capture rates were based on eight trap-nights for chemical lures and ten for live mice. Live mice were used as a positive control and the effectiveness of each lure was measured by capture rate per treatment. In this and all trapping experiments, captured snakes were removed from the area.

In a second experiment on Guam in May of 1997, 80 ml of 0.5% putrescine was wicked into absorptive cotton and then placed into traps in the forest adjacent to Tarague Beach. Seven traps of each treatment type (live mouse, putrescine, empty trap) were run for two nights resulting in 14 trap-nights/treatment.

In a third experiment, we used permeation tubes (HRT type, Kin-tek, Laurel, TX) to slowly release the sulfur compounds ethanethiol, dimethyl disulfide, and dimethyl sulfide from within traps. We placed permeation tubes (one tube of each chemical) within black-felt covered lure holders within each brown tree snake trap. We set a series of trap-lines adjacent to Scout Beach during August 1997 to examine the attractiveness of these chemicals. We set three chemical lure traps, three live mouse lure traps, and three empty control traps in four trap-lines. Trap-lines were moved daily for four nights to produce 12 trap-nights per treatment.

For our fourth experiment, we used singly presented sulfur compounds as lures. Trap-lines were run adjacent to Scout Beach Guam during August of 1997. We set three traps of each treatment type. The treatments were ethanethiol, dimethyl disulfide, dimethyl sulfide (in permeation tubes), live mice, and empty control traps. Trap-lines were moved daily for five nights to produce 15 trapnights per treatment.

### 3. RESULTS

In the first experiment, four snakes were captured with live mice (40% capture rate), but none were captured with the chemical treatment. The dimethylamine, trimethylamine and cadaverine treatment was much less successful in capturing brown tree snakes than live mice ( $F_{1,16} = 4.74$   $P = 0.045$ ).

In the second experiment, ten snakes were captured with live mice (71% capture rate), one snake was captured with putrescine, and one with an empty trap (7% capture rate). Putrescine was of very limited effectiveness for capturing brown tree snakes compared to live mice ( $F_{2,39} = 15.955$ ;  $P < 0.001$ ). The capture rate of putrescine was 10% that of live mice in traps, and it was not different than the capture rate using empty traps (Figure 1).

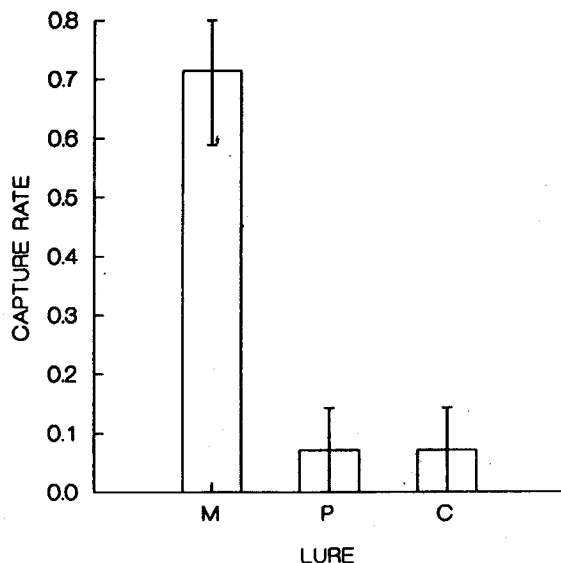


Figure 1. Capture rate by lure provided for brown tree snakes on Guam based on 14 trap-nights per treatment. M = live mouse, P = putrescine soaked cotton, C = empty, clean trap control. Bars represent one standard error.

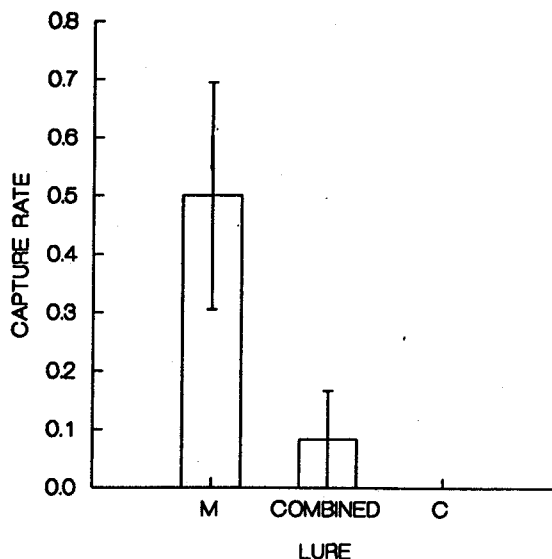


Figure 2. Capture rate by lure provided for brown tree snakes on Guam based on 12 trap-nights per treatment. M = live mouse, Combined = ethanethiol, dimethyl disulfide, and dimethyl sulfide presented simultaneously, C = empty, clean trap control. Bars represent one standard error.

In the third experiment, using ethanethiol, dimethyl disulfide, and dimethyl sulfide compounds simultaneously was not as effective as live mice in luring brown tree snakes into traps ( $F_{2,33} = 0.015$ ; Figure 2). We captured one snake in 12 trapnights. This capture rate was 16% as effective as live mice, with which we captured six snakes (50% capture rate; Tukey test,  $P = 0.055$ ).

In the fourth experiment, when the compounds were presented individually, differences were more striking ( $F_{4,70} = 12.923$ ;  $P < 0.001$ ). Twelve snakes were captured with mice (80% capture rate), but none were captured with ethanethiol, dimethyl disulfide, dimethyl sulfide or in the control traps.

#### 4. DISCUSSION

The purpose of this study was to make an initial evaluation of the effectiveness of odors normally associated with decomposition for attracting brown tree snakes. We must eschew broad inference from our results because although the compounds we tested were not attractive in these pilot studies, one of these compounds could still be an essential component of an attractive chemical stimulus. Our results firmly suggest, however, that a simple chemical attractant is unlikely to be found by examining individual compounds perceived by humans as important components of the odor of decomposition. More comprehensive testing is required before stronger inference can be made as to the influence these compounds have on appetitive foraging behavior of brown tree snakes, but we do not believe that these compounds will prove useful as artificial lures.

We caution undue optimism regarding the quick discovery of simple chemical signals for an artificial lure system, especially if the candidate lures are compounds that are selected on the basis of human perception. Human perception is likely to be an inappropriate basis for sensory studies of other animals. For example, many procellariiform birds are attracted to the odors of a variety of fish oils and fish by-products (Clark and Shah, 1992). To humans, these food items have a distinctly "fishy" smell largely attributable to the

methylamine compounds. However, when fractions were prepared from krill and field tested for attractiveness using Leach's Storm Petrels (*Oceanodroma leucorhoa*), the carboxylic acid extracts were the most attractive component of krill. Extracts dominated by phenols or amines were less attractive, and in fact, did not statistically differ from the negative control (water) in their ability to lure petrels to a target (Clark & Shah 1992). Thus, the compounds which signal "krill" to humans are substantially different than the compounds that signal "krill" to petrels. Similarly, compounds salient to humans do not seem to be salient to brown tree snakes, based on the experiments reported here.

We know that whole odors of decomposition are attractive to brown tree snakes, but that various amine and mercaptan components of carrion odors do not appear to be (Clark, in press). Testing individual odors is not an efficient method of lure development. Nearly 10 years of this inductive approach has failed to produce a strong candidate lure. For future studies, we urge a more systematic method to identify effective components for lures. A standard fractionation and assessment for biological activity is required. Researchers should concentrate on serially fractionating carrion odors until salient components can be deduced rather than taking a "shotgun" approach, hoping that one snake-attracting chemical may serendipitously be found.

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